ABSTRACT

The present invention provides an entirely new method for mutagenesis, which is simple, speedy, economical, and widely-applicable.

A method for mutagenesis comprising steps of:

DNA synthesis in which primers which have mutations and a phosphorylated 5'-terminus are annealed to a template DNA and then subjected to an elongation reaction using a thermostable high-fidelity DNA polymerase, after which the phosphorylated 5'-terminus and the elongated terminus are ligated by means of a thermostable DNA ligase to synthesize a circular DNA containing said primers;

digestion in which at least DNAs other than the amplified circular DNA are digested into several fragments; and

double-stranded DNA synthesis in which, with the several fragments obtained in the above step of digestion as megaprimers, said megaprimers are annealed to said circular DNA synthesized in the above step of DNA synthesis, followed by an elongation reaction performed using said thermostable high-fidelity DNA polymerase.